IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Masako Kimura et al. :

Serial No. 09/076,124 : Group Art Unit: 1614

Filed on May 12, 1998 : Examiner: Z. Fay

For: COMPOSITIONS CONTAINING DIFLUPREDNATE

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of
Patents and Trademarks,
Washington, D.C. 20231

Sir:

I, Kenichi Haruna, residing at 443-60, Inokuchi, Himeji-shi, Hyogo 670-0983 Japan, sincerely declare;

That my education and employment history is as follows:

I graduated from Department of Biotechnology, Faculty of Engineering, Okayama University, Okayama, Japan, in March of 1991,

I graduated from Department of Biotechnology, Graduate School of Engineering, Okayama University, Okayama, Japan, in March of 1993,

I have been engaged in researches at Senju Pharmaceutical Co., Ltd. since April of 1993, mainly in the field of immunology and allergology;

That the following experiments were conducted under my direction and supervision, and the experiment demonstrated that an emulsion containing difluprednate of the present invention exhibited superior antiinflammatory action as compared to a suspension containing difluprednate of US 5,556,848, the results of which follow hereunder;

Experiments

The emulsion containing difluprednate of the present invention and the suspension containing difluprednate of US 5,556,848 were compared for the antiinflammatory action in rat endotoxin-induced uveitis (hereinafter EIU) models.

Test 1: Effects on rat EIU

1. Test animal

Male Lewis rats weighing about 150 g

2. Test drugs

(1) emulsion containing 0.05% difluprednate (0.05% DP-Em, median size 0.18 μm)
(2) emulsion containing 0.01% difluprednate (0.01% DP-Em, median size 0.16 μm)
(3) suspension containing 0.05% difluprednate (0.05% DP-Sus, median size 7.1 μm)
(4) saline

3. Test method

A solution of 2 mg/ml endotoxin (derived from *Salmonella minnesota*, Sigma) dissolved in saline was injected into the foot pad of the rats by 100 μl to induce uveitis. After 20 hours, the rats were slaughtered and the aqueous humor of the both eyes was collected. The protein concentration and the leukocytes in the aqueous humor were measured and used for the evaluation of uveitis state. Each drug was instilled into the both eyes by 5 μl immediately after injection of endotoxin and 6 hours later.

4. Results

The results are shown in Fig. 1 and Fig. 2.

The 0.05% emulsion exhibited stronger inflammation inhibitory effect, as evidenced by the both indices of protein concentration and leukocyte counts, than the 0.05% suspension, and there was found statistically significant difference between the two groups.

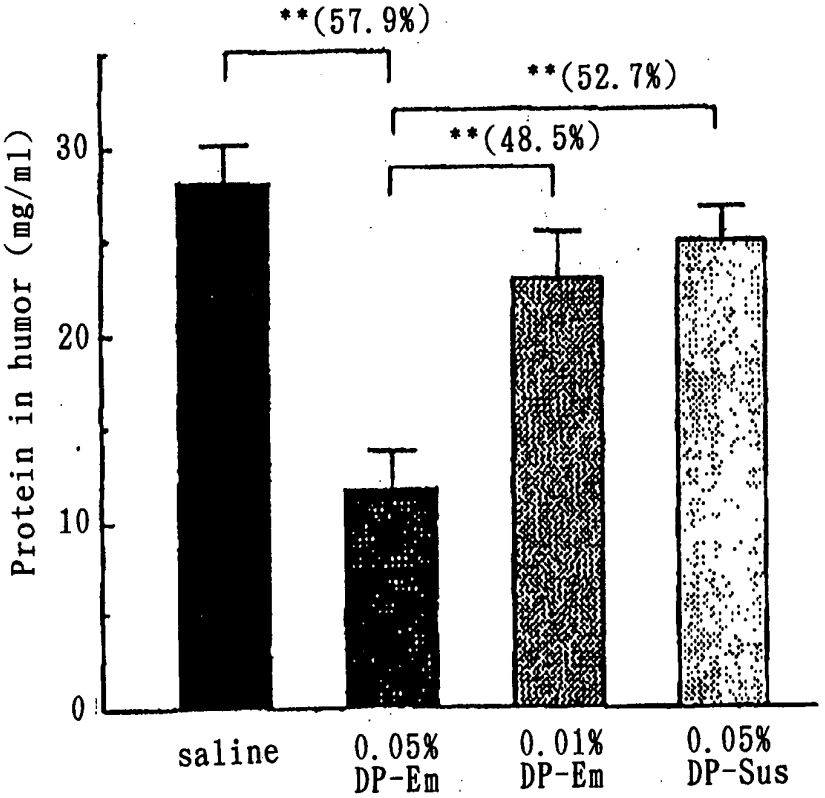

Fig. 1 Effects of difluprednate (DP) on protein content in aqueous humor in EIU in rat. Each column represents mean±S.E. n=9 **:p<0.01(Dunnett test; 2-side)

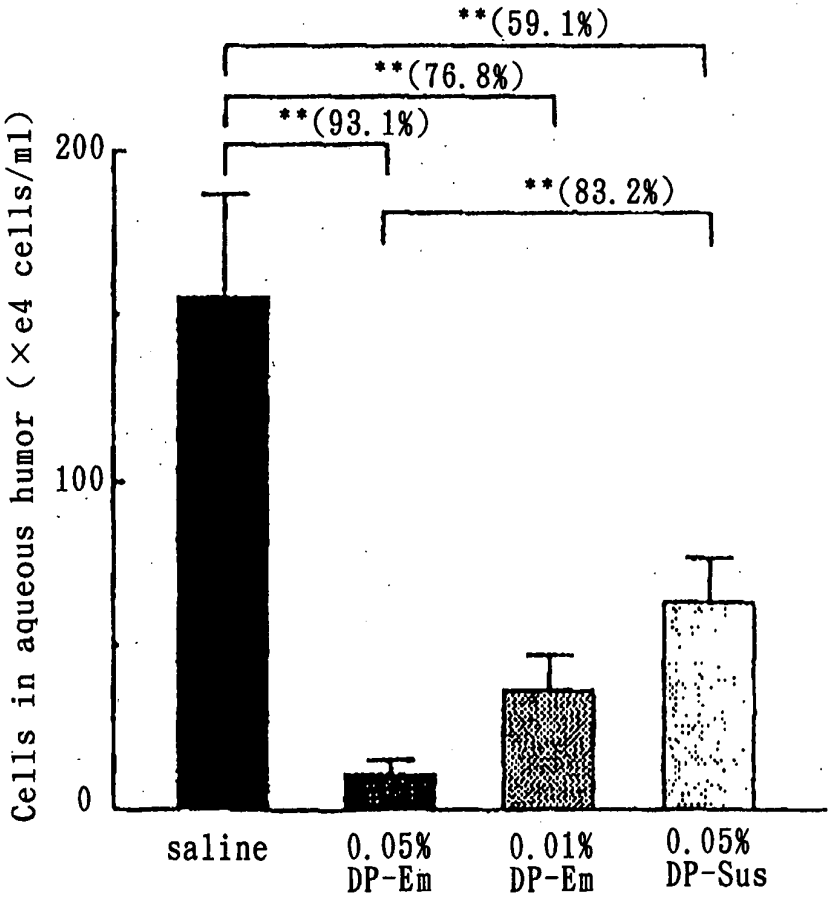

Fig. 2 Effects of difluprednate (DP) on infiltration of inflammatory cells to aqueous humor in EIU in rat. Each column represents mean±S.E. n=9 **:p<0.01 (Dunnett test; 2-side)

Test 2: Effects on rat EIU

1. Test animal

Male Lewis rats weighing about 170 g

2. Test drugs

(1) emulsion containing 0.05% difluprednate (0.05% DP-Em)
(2) suspension containing 0.1% difluprednate (0.1% DP-Sus)
(3) saline

3. Test method

A solution of 2 mg/ml endotoxin (derived from *Salmonella minnesota*, Sigma) dissolved in saline was injected into the foot pad of the rats by 100 μl to induce uveitis. After 24 hours, the rats were slaughtered and the aqueous humor of the both eyes was collected. The protein concentration of the aqueous humor was measured and used for the evaluation of uveitis state. Each drug was instilled into the right eye by 5 μl immediately after endotoxin injection and 4 and 8 hours later.

4. Results

The results are shown in Table 1.

The 0.05% emulsion and 0.1% suspension both exhibited significant inflammation inhibitory effect on rat EIU. Despite the lower difluprednate concentration of the 0.05% emulsion, it showed stronger inflammation inhibitory effect than the 0.1% suspension, and there was found statistically significant difference between the two groups.

Table 1

Drug	Protein in aqueous humor (mg/ml)		Inhibition (%)
Saline	24.3 ± 2.4		—
0.05% DP-Em	6.9 ± 3.6*	$p<0.01$	71.6
0.1% DP-Sus	18.1 ± 2.9**		25.5

Each value represents mean ± SD. Significant difference from saline treated group: *;$p<0.05$, **;$p<0.01$ (Dunnett's test, n=5)

The test drugs used in the above tests were prepared by the following method and had the compositions shown in Table 2.

Preparation of 0.05% and 0.01% difluprednate-containing emulsions

Sterile purified water was heated to about 70℃ and polysorbate 80, concentrated glycerol, sorbic acid, sodium acetate, boric acid and sodium edetate were added and dissolved. Its pH was adjusted to 5.5 with sodium hydroxide to give an aqueous phase. Separately, castor oil was heated to about 70℃ and a predetermined amount of difluprednate was added and dissolved to give an oil phase. The oil phase was added while stirring the aqueous phase with a homomixer to give a crude emulsion. This crude emulsion was finely divided in a microfluidizer and sterilized by filtration to give a difluprednate-containing emulsion.

Preparation of 0.05% and 0.1% difluprednate-containing suspensions

Sterile purified water was heated to about 80℃ and hydroxypropylmethylcellulose (60SH50) was added. The mixture was thoroughly stirred and cooled to room temperature to dissolve hydroxypropylmethylcellulose (60SH50). Thereto were added sodium acetate, sodium chloride and benzalkonium chloride and dissolved. The solution was adjusted to have pH 5.0 with hydrochloric acid and sterilized by filtration. A predetermined amount of difluprednate was added and dispersed by ultrasonication to give a difluprednate-containing suspension.

Table 2

	0.05% DP-Em	0.01% DP-Em	0.1% DP-Sus	0.05% DP-Sus
Difluprednate	0.05 g	0.01 g	0.1 g	0.05 g
Castor oil	5.0 g	5.0 g	—	—
Polysorbate 80	4.0 g	4.0 g	—	—
Concentrated glycerol	2.2 g	2.2 g	—	—
Sorbic acid	0.1 g	0.1 g	—	—
Boric acid	0.1 g	0.1 g	—	—
Sodium edetate	0.02 g	0.02 g	—	—
Sodium acetate	0.05 g	0.05 g	0.1 g	0.1 g
Sodium chloride	—	—	0.8 g	0.8 g
HPMC (60SH50)	—	—	0.2 g	0.2 g
Benzalkonium chloride	—	—	0.005 g	0.005 g
HCl	—	—	q.s.	q.s.
NaOH	q.s.	q.s.	—	—
total amount (ml)	100	100	100	100
pH	5.5	5.5	5.0	5.0

HPMC: hydroxypropylmethylcellulose

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Hyogo, Japan on this 7th day of December, 1998

Kenichi Haruna

Kenichi Haruna